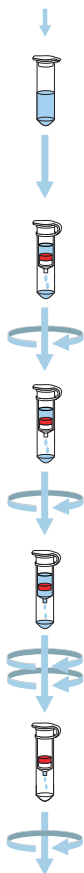


GENELUTE BLOOD GENOMIC DNA KIT

Whole Blood



Pure Whole Blood Genomic DNA

1 Release DNA from fresh or aged whole blood

- Collect whole blood in an anticoagulant tube & equilibrate to RT.
- Place 20 μ l of Proteinase K in a 1.5 ml microcentrifuge tube. Add up to 200 μ l of whole blood (or blood + Resuspension Solution to 200 μ l). *Optional: Add 20 μ l RNase. Mix & incubate at RT, 2 min.*
- Add 200 μ l of Lysis Solution. Vortex or pipet to mix.
- Incubate at 55 $^{\circ}$ C, 10 min.

2 Prepare column

- Add 500 μ l Column Preparation Solution to binding column.
- Spin at $\geq 12,000 \times g$, 1 min. Discard flow-through.

3 Bind DNA to column

- Add 200 μ l ethanol to lysed blood. Vortex or invert to mix.
- Transfer to binding column. Spin at $\geq 6,500 \times g$, 1 min.

4 Wash to remove contaminants

- Transfer column to new collection tube. Add 500 μ l of Prewash Solution or Wash Solution to column. Spin at $\geq 6,500 \times g$, 1 min.
Note: Ethanol must be added to Prewash Solution or Wash Solution concentrate before first use.
- Transfer column to new collection tube. Add 500 μ l of Wash Solution to column.
- Spin at $\geq 12,000 \times g$ for 3 min. to dry column.

5 Elute purified DNA

- Transfer column to new collection tube.
- Add 200 μ l Elution Solution. Spin at $\geq 6,500 \times g$, 1 min. *Optional: Repeat elution in same or new tube.*



Please refer to the technical bulletin for additional information.

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Problem	Cause	Solution
Binding column is clogged	Sample is too large	In the future, use a smaller quantity of whole blood. To salvage the current preparation, increase the <i>g</i> -force and/or spin longer until the lysate passes through the binding column. The yield of genomic DNA may be reduced.
Poor or low genomic DNA recovery	Sample is old or degraded	The yield will vary among individual samples of fresh or aged whole blood. Use whole blood within a few hours of obtaining the sample for best results. If samples are being stored for future use, whole blood may be kept at 4 °C for at least 3 months.
	Lysate/ethanol mixture is not homogeneous	To ensure a homogeneous solution, vortex 5-10 seconds before applying to the binding column. If minimally sheared genomic DNA is desired in downstream applications, e.g. if using the end product for long amplification PCR, mix with gentle pipetting or inversion until homogeneous instead of vortexing.
Purity of the DNA is lower than expected; A_{260}/A_{280} ratio is too low	DNA elution is incomplete	Confirm that the DNA was eluted in 200 µl of Elution Solution. DNA yield for most types of material may be improved by incubating the Elution Solution for 5 minutes at room temperature after it is added to the column. A second and third elution using 200 µl of Elution Solution may be performed.
	Ethanol was omitted during binding	Check that the ethanol was added before applying the sample to the binding column.
	The eluate contains residual ethanol from wash	Ethanol from the final wash must be eliminated before eluting the DNA. Spin longer or a second time to dry the membrane. If flow-through liquid containing ethanol contacts the binding column, repeat the centrifugation step before eluting DNA.
	Prewash and/or Wash Solution concentrate were not diluted before use	Check that Prewash Solution and Wash Solution concentrate were properly diluted with ethanol before use.
Purity of DNA is lower than expected; A_{260}/A_{280} ratio is too high	Water was used for elution instead of Elution Solution	Elution Solution is recommended for optimal yields and storage of the purified DNA. If water is used to elute the DNA, confirm that the pH is at least 7.0, to avoid acidic conditions which may subject the DNA to acid hydrolysis when stored for long periods of time.
	Eluate was diluted in water for absorbance measurement	Use either Elution Solution (10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0) or 10 mM Tris-HCl, pH 8.0-8.5 as the diluent.
	Background reading is high due to silica fines	Spin DNA sample at maximum speed for 1 minute; use the supernatant to repeat absorbance readings.
DNA is sheared	Blood sample was older than 24 hours	In future preparations use the Prewash Solution in the first wash step.
	Purification is incomplete due to column overloading or inadequate lysis	Reduce the initial volume of blood or increase the lysis time while monitoring the lysis visually.
Downstream applications are inhibited	Genomic DNA is contaminated with RNA	Include an RNase A treatment step in future isolations or treat final product with RNase A Solution and repurify.
	Genomic DNA was handled improperly	This kit is designed to eliminate DNA precipitation and resuspension, common steps found in other genomic DNA kits that can lead to shearing. All pipetting steps should be executed as gently as possible. Wide-orifice pipet tips are recommended to help eliminate shearing. If minimally sheared genomic DNA is desired in downstream applications, e.g. if using the end product for long amplification PCR, mix with gentle pipetting or inversion until homogeneous instead of vortexing.
Downstream applications are inhibited	Blood sample is old, degraded, or has undergone repeated freeze/thaw cycles	Old starting material may yield degraded DNA in the eluate. For best results, fresh whole blood preparations should be used immediately. Alternatively, whole blood can be stored at 4 °C for up to 3 months.
	Ethanol is carried over into the final genomic DNA preparation	After the final wash of the binding column, do not allow the flow-through liquid to contact the column. Re-spin the column for an additional 1 minute at maximum speed (12,000-16,000 <i>x g</i>), if necessary, after emptying the collection tube.
	Salt is carried over into the final genomic DNA preparation	Make sure that the binding column is transferred to a new 2 ml collection tube before adding Prewash or Wash Solution.